long-term protection and promotion of human health the basis for their environmental policies.

Immunization campaigns were stressed in another United States co-sponsored resolution on the WHO expanded program on immunization. The Director General was requested to intensify activities pertaining to the development of immunization programs especially for the developing countries, as well as to continue support of research on the efficacy of vaccines. It is noted that in extensive regions of the world, immunization is still available for only a small portion of the children in the susceptible age groups.

The discussion of the onchocerciasis (river blindness) program was most interesting since it is estimated that among the 10 million inhabitants of the area of the Volta River basin in Africa, more than one million are infected with onchocerciasis and, of that million, at least 70,000 are considered economically blind and many more suffer visual impairment. This disease, carried by the black fly, constitutes the most important deterent to human settlement and development of the fertile valleys of the Volta River basin which are now essentially uninhabited and nonproductive. Since there is still no drug suitable for mass chemotherapy, a massive multilateral aid program has been instituted and is scheduled to begin in November 1974. This is a 20-year effort to eliminate river blindness, and a resolution was adopted urging the countries that provide bilateral and multilateral aid through special health programs to consult with WHO for best coordination of effort.

It is necessary to stress the great relevance of the role of WHO to the practicing physician as it affects him in daily practice due to the increasing mobility of the patient population in the jet age. There are many examples of this relevance, including immunization, the possible global eradication of various diseases, especially smallpox, the epidemiologic aspects of cancer, and the global aspects of venereal disease.

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Notes from Important or Unusual Meetings

Conference on the Pathophysiology of Diabetes Mellitus

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A WESTERN REGIONAL CONFERENCE on diabetes mellitus was held at The Kroc Foundation in the Santa Ynez Valley of California, November 13-16, 1973. The purpose of the conference was to bring together some of the persons active in diabetes research in the western United States. Twenty-four investigators attended the conference and discussed papers given by 13 of them. In addition two brief presentations were made. This report summarizes the 15 presentations for those interested in current western diabetes research activities.

The first was by Peter H. Bennett, MD, acting chief of the Southwestern Field Studies Section, National Institute of Arthritis and Metabolic Diseases, Phoenix, Arizona. He brought up to date the information about continuing work on diabetes in Pima Indians. The Pimas have the highest prevalence of diabetes of any population in the world. Essentially half of all Pimas beyond middle age have diabetes as measured by glucose intolerance. Extensive observations have been made on the Pimas for this reason, and Dr. Bennett described the relation between serum insulin and glucose levels after a glucose load. The immunore-

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active insulin (IRI) level two hours after a glucose load was analyzed. Between glucose levels of 80 and 160 mg per deciliter the IRI rose as glucose rose, but above 160 mg per deciliter of glucose it fell as glucose increased. The same biphasic relationship between IRI and glucose has been observed in a study by Eschwege on Paris policemen. Although obesity increases IRI at all two-hour glucose levels, it has no effect on the position of the IRI maximum at 160 mg per deciliter glucose. Dr. Bennett next examined long-term studies of insulin response to ascertain whether specific IRI response patterns preceded glucose tolerance. Deteriorating glucose tolerance was preceded by higher two-hour IRI levels, but both phenomena were seen more in overweight Pimas. Obesity complicated the analysis, for it was associated with both future deterioration of glucose tolerance and higher two-hour serum insulin level. When deteriorating glucose tolerance of non-obese persons was compared with stable glucose tolerance of non-obese persons, no IRI response difference could be demonstrated. Dr. Bennett concluded that no abnormality of insulin production could be demonstrated to be present before diabetes develops.

Maurice E. Krahl, PhD, professor and chairman, Department of Physiology of Stanford University School of Medicine, briefly described his observations on fragmented growth hormone. He has been examining the possibility that a product of growth hormone might inhibit glucose utilization and hence be responsible for the hyperglycemia seen several hours after growth hormone administration. A fragment obtained from human and sheep growth hormones of less than 1,800 daltons molecular weight produced hyperglycemia in the ob/ob mouse. The fragment itself is probably from the C-terminal area of the intact growth hormone molecule.

Mayer B. Davidson, MD, assistant professor of medicine of University of California, Los Angeles, School of Medicine, described his studies of the effect of insulin and glucagon on glucose metabolism in rat liver. Using liver slices in a high potassium buffer, he demonstrated that insulin has several effects. It increases glycogen generation from either glucose or glycerol, in the medium, and accelerates lipid synthesis from glycerol. It inhibits gluconeogenesis from increased alanine, glycerol, or fructose. Insulin produces a simultaneous decline in glucose release and an increase in glycogen production; the conversion of radioactive glycose to carbon dioxide is unaffected. Glucagon effects were studied using liver slices in a low potassium buffer. Glucagon did not counteract the inhibition of gluconeogenesis produced by insulin. On being informed that glucagon content was monitored by radioimmunoassay, Dr. Unger stated that it was questionable whether glucagon detected by immunoassay in an *in vitro* study was still biologically active. After the conference Dr. Davidson confirmed the biologic activity of glucagon in his system by demonstrating that glucagon did produce glycogenolysis in liver slices from fed rather than fasted rats. He concluded that glucagon does not compete with the direct inhibition of gluconeogenesis by insulin.

Daniel Porte, Jr., MD, professor of medicine and chief of the Endocrinology Section, University of Washington School of Medicine, presented studies of neural influences on insulin secretion. He demonstrated the role of the sympathetic nervous system, first showing that the beta adrenergic agent, isoproterenol, can stimulate early insulin release even in patients who do not respond to glucose. Propranolol, the beta adrenergic blocker, stopped isoproterenol-induced insulin rise, but had no effect on glucose-mediated insulin elevation. He reported a patient with grossly abnormal carbohydrate tolerance whose plasma insulin initially fell rather than rose when glucose was given. Infusion of the alpha adrenergic blocker, phentolamine, resulted in improved glucose tolerance and a restoration of the early phase of insulin release. He then discussed the role of the central nervous system in insulin secretion. Rats given injections of insulin or tolbutamide became hypoglycemic. If the injections are repeated several days in a row, the animals will become hypoglycemic even if saline solution rather than insulin is injected. This conditioned response apparently involves both the pancreas and vagus nerve since it is abolished by streptozotocin or vagotomy. Atropine also abolishes the hypoglycemia, suggesting that the parasympathetic nervous system is involved. A central mechanism modifying insulin production was suggested by additional studies. Pancreatic insulin release and hypoglycemia were produced by injection of normal but not denatured insulin into the cerebral spinal fluid. Vagotomy blocked the response. Dr. Porte proposed that these observations could be explained by a central nervous system area which responds to changes in glucose metabolism by causing insulin release.

R. Philip Eaton, MD, associate professor of medicine, University of New Mexico School of Medicine, discussed glucagon and insulin secretion in glucose-fed rats and cobalt-treated rats. Animals fed 60 percent glucose in saline solution as their total diet for one week were found to have improved glucose tolerance even though blood insulin levels were lower. In the liver of glucose-fed rats, levels of several metabolic intermediates were found to have a pattern like that produced by high insulin levels in other circumstances. Dr. Eaton concluded that this similarity was due to increased liver sensitivity to insulin. This concept was supported by his observations of phosphoenolpyruvate carboxykinase levels in these animals. The glucose-fed rat retained a normal hyperglycemic response to administered glucagon, but it had a low endogenous blood glucagon level. Arginine administration produced glucose, insulin and glucagon responses below those of controls. Experiments carried out in colbalt-treated rats were then described. Glucose tolerance remained similar to that in controls but insulin levels decreased, free fatty acids rose, and liver phosphoenolpyruvate fell. Glucagon administration did not produce a normal blood glucose rise and did not cause insulin release, but its injection did change the levels of glucose intermediates in the liver and increased the rate of glycogenolysis. In cobalt-fed rats, arginine produced greater hormone responses than in the controls. Dr. Eaton speculated that diet or hormonal agents may change liver sensitivity to insulin through effects on the activity of gluconeogenic enzymes.

Roger H. Unger, MD, professor of internal medicine of the Texas Southwestern Medical School and the Dallas Veterans Administration Hospital, proposed glucagon and insulin as major factors controlling pathways of metabolism in the face of a varying dietary pattern. Glucagon opposes the action of insulin, for it is produced when no food is ingested, while insulin is produced when food is consumed. Levels of the two hormones vary in a reciprocal manner effectively represented by the insulin-glucagon ratio. The ratio may rise to as high as 70 to 1 after a high carbohydrate meal, but in starvation it falls to below 1 to 1. Glucagon levels have also been observed to increase when plasma volume falls. Ketoacidotic diabetic patients have very high glucagon levels, in part triggered by the decline in plasma volume in this condition. The high glucagon levels probably produce the increased numbers of hepatic lysosomes seen in ketoacidosis. Diabetic patients, even under good control, have increased fasting glucagon levels, poor suppression of glucagon by hyperglycemia, and excessive glucagon response to amino acids. Negative nitrogen balance develops in insulin controlled diabetic dogs and they produce large amounts of urea when given small amounts of glucagon. In conclusion, Dr. Unger speculated that lowering glucagon levels by pharmacologic means such as somatostatin might be beneficial to diabetics. In discussion, Dr. Rachmiel Levine pointed out that neither glucagon nor insulin is absolutely required for metabolic alteration; he cited the Houssay animal as evidence. Dr. Unger indicated that some enteroglucagon might be produced by Houssay animals, for it is found in pancreatectomized humans. He recalled human cases where no glucagon was measurable and agreed with Dr. Levine that the two hormones are tonal in character and not absolutely necessary. He asserted that insulin and glucagon are essential for metabolic flexibility in a changing environment.

Paul M. Beigelman, MD, associate professor of medicine and pharmacology, University of Southern California School of Medicine, reported on the release of insulin from isolated single islets. He reviewed the morphologic aspects of insulin release demonstrated by electron microscopy. Islets stimulated by high glucose levels develop increased rough endoplasmic reticulum, fusion of cytoplasmic vacuoles, and hypertrophy of the Golgi apparatus. He studied the release of insulin in a zero glucose medium and found that healthy islets released less than 15 microunits of insulin in a 15 minute period. A small amount of insulin was regularly detectable suggesting low level insulin secretion in the absence of glucose. Glucose stimulation resulted in more than a doubling of insulin production in almost all islets studied. A new technique was developed to measure insulin production by single islets. A "dip-stick" pipette was used to immerse one islet for 15 seconds into each of a series of wells on a rotating table. Results were preliminary, but suggested that insulin secretion is pulsatile in character, with an average oscillation period of 60 seconds in glucose-free medium and 42 seconds in 300 mg per deciliter of glucose. Oscillation might be an important factor in insulin secretion, each islet functioning as a physiological syncytium.

Karl E. Sussman, MD, associate professor of medicine of the University of Colorado Medical

Center, and chief of the Medical Service of the Denver Veterans Administration Hospital, described his studies on the association of adenine nucleotides and insulin release. In the adrenal medulla it is known that exocytosis of hormone-bearing chromaffin granules is associated with adenosine triphosphate (ATP) release. Dr. Sussman used isolated rat islets in perifusion studies and was able to demonstrate an abrupt rise in ATP in the medium when insulin was released following a 300 mg per deciliter of glucose challenge. The rise in ATP and the amount of insulin released were correlated. Radioactive percursors of ATP were used to determine whether the release of ATP and insulin occurred simultaneously. Radioactive ATP was released with insulin from glucose-stimulated islets when incubation with radioactive adenine had been prolonged sufficiently to label the insulin granules. An effort was also made to identify the source of the released ATP. Using sodium fluoride and Antimycin A, Dr. Sussman showed that insulin release could be blocked without total depletion of islet ATP. He concluded that two ATP pools were present in beta cells. Mitochondrial ATP apparently supplies energy for insulin release to the other pool in the beta granules which then is unaffected by mitochondrial poisons and is released with insulin.

Gerold M. Grodsky, PhD, professor of biochemistry of the University of California, San Francisco, School of Medicine, described work he and Miss Barbara Frankel did on the perfused pancreas of Chinese hamsters. In this animal, as in man, diabetes develops in a pattern indicating genetic transmission. Nine generations of glycosuric brother-sister matings produced hamsters which were homozygous for 90 percent of the genetic traits examined. These animals also showed no intragroup skin transplant rejection. Non-ketotic animals were mated and 20 percent of the offspring showed ketosis and diabetes, 50 percent were non-ketotic and diabetic, 10 percent had minimal glycosuria, and the remaining 20 percent were aglycosuric, but had a high frequency of glucose intolerance. Orci, in Geneva, has found that in the pancreatic islets of diabetic Chinese hamsters alpha cells are increased in number. Studies were done to compare the responses of the isolated perfused pancreas of non-ketotic diabetic Chinese hamsters to aglycosuric littermates. The studies showed that following a glucose challenge, the two phases of insulin release occurred in the normal time pattern, but were only half as great in the diabetic animals as in the controls. The pancreas of diabetic hamsters also had higher baseline glucagon levels, which were poorly suppressed by high perfusate glucose concentrations. In the diabetic pancreas arginine was a poor stimulator of insulin release, but a strong stimulator of glucagon release. Theophylline produced similar insulin and glucagon responses in both diabetic and aglycosuric pancreas. Though the pancreas of some diabetic animals had either a normal insulin or glucagon response to glucose, in none were both hormone responses normal. The insulin-glucagon ratio following glucose was found to separate the responses of islets from diabetic hamsters almost completely from those of controls.

Rachmiel Levine, MD, executive medical director, City of Hope, Duarte, California, briefly described current work by Robert Seecof, of that institution, on the effect of insulin on muscle and myoneural cell cultures of drosophila, the fruit fly, an invertebrate organism which does not produce insulin. Insulin added to these cultures produced cell growth, cell proliferation, and increased cell fat. A substance immunologically similar to pork insulin was found in larval drosophila tissue. A structural similarity between insulin and vertebrate nerve growth factor, a locally active substance important in embryonic development, has also been observed. In discussion, the possibility that these three substances could be evolutionary products of a primitive growth-related material was mentioned.

Edward R. Arquilla, MD, PhD, professor and chairman of the Department of Pathology, University of California, Irvine, California College of Medicine, related the structure of the insulin molecule to its biologic function. The structural detail of the insulin molecule has been determined by using x-ray diffraction. Crystalline insulin is a hexamer consisting of three dimers. In solution, insulin is usually monomeric. Dimerization occurs only at concentrations greater than 50 μg per ml and involves a specific surface of the insulin molecule. Previous molecular modification studies have shown that the small size of the N-terminal amino acid of the A-chain (glycine) and the area of the B-chain adjacent to tyrosine A-19 are important to insulin's biologic function.

Dr. Arquilla reported additional information about insulin structure-function relations gained using immunologic techniques. Insulin may be measured by using immune hemolysis as well as

by the usual insulin radioimmunoassay. In immune hemolysis, stable insulin competes with insulin-coated erythrocytes for anti-insulin antibodies which, if attached to the erythrocytes, induce hemolysis. It has been found that a number of insulin derivatives will react quite differently with the same antiserum in the two systems, indicating that the antibodies important to the two assays react to separate portions of the insulin molecule. Radioimmunoassay antibodies appear to be directed predominantly to the area of the A-chain involved in dimerization. Antibodies important to the immune hemolysis system are directed to other areas of the insulin molecule. Antibodies to insulin derivatives have been shown to have unique properties in these assays. Antisera to desalanine-desasparagine insulin, a biologically inactive derivative, react in the radioimmunoassay and not in immune hemolysis. They have been found to neutralize insulin *in vivo* better than antisera to insulin itself, suggesting that the dimerization site may be involved in insulin's biologic activity.

The invited lecturer, David M. Kipnis, MD, chairman of the Department of Medicine of Washington University School of Medicine in St. Louis, spoke on his studies of hypoglycemia, the autonomic nervous system, and insulin production by the pancreas. He first reported studies on a group of reactive hypoglycemic patients in whom late hypoglycemia regularly developed but whose glucose tolerance was otherwise within normal limits. These persons regularly had high 30-minute and 60-minute serum glucose levels, suggesting rapid glucose absorption from the stomach. Indium 113 was used to examine their rate of gastric emptying, which was found to be normal. However, when glucose was instilled directly into the duodenum rather than ingested, their serum glucose rise was more rapid than that of normal controls. Moderate doses of phenformin stopped this rapid serum glucose rise and also reduced the insulin response and prevented the late hypoglycemia which would otherwise occur. It was not clear whether phenformin changed the secretion of insulin or reduced the rate of glucose uptake.

Cholinergic blockade was also effective in treatment of reactive hypoglycemia. An unusual patient with long-standing symptomatic postprandial hypoglycemia had a strikingly elevated insulin response to oral glucose which became normal following propantheline. In fasting, the insulin response to a glucose load is characteristically blunted. A small amount of food preceding the load will restore the insulin response to normal. An inducible glucoreceptor substance in the beta cells is one explanation of this phenomenon. Beta methylglucoside and phloridzin (phloretin-betaglucoside) have been found to stimulate insulin secretion in isolated rat islets. The structural similarity of these substances is further evidence for a glucoreceptor. Lectins are plant proteins which react with substances on animal cell surfaces. Of a large group of plant lectins, only mushroom phytohemagglutinin (PHA) effectively stimulated insulin secretion. It also produced glucagon release from the pancreas and growth hormone release from the pituitary. Mushroom PHA binds to a specific glycopeptide on the cell surface. Its half saturation binding concentration, 2.9×10^{-7} moles per liter, is the same as that required for it to produce half-maximal insulin release. A mushroom PHA concentration sufficient to stimulate insulin release did not increase conversion of glucose to carbon dioxide; calcium was not required for its action. Mushroom PHA appears to be a useful new tool for studying membrane phenomena involved in the secretory process.

John W. Farquhar, MD, associate professor of medicine, Stanford University School of Medicine, discussed the relation between atherosclerosis and diabetes. There is an increased prevalence of coronary heart disease in both males and females whose response to a glucose load is *above* the 80 percentile for their age. Other kinds of vascular diseases and blood pressure elevation are also associated with marginal glucose tolerance. Blood lipid elevation may contribute to the observed relation between atherosclerosis and diabetes. In patients with severe diabetes, when blood insulin is low, a decline in lipoprotein lipase produces increased serum lipid levels. A different situation is present in mild diabetes. Overproduction, rather than impaired clearance, produces elevated lipid level. Obesity has an effect unrelated to blood sugar level. In childhood-onset obesity, there is a pronounced increase in the body's total number of fat cells. In adult-onset obesity, fat cell number does not change, so that fat cell size is often decidedly increased. The oversized adipocyte is particularly resistant to insulin action. This insulin resistance may generate a hyperinsulinism which in turn leads to overproduction hyperlipemia. Weight reduction causes diminution in adipose cell size, leading to

reduced insulin resistance and decline in plasma triglyceride and cholesterol levels. Predilection to atherosclerosis may well be decreased by the fall in insulin level associated with weight loss.

Gerald M. Reaven, MD, professor of medicine, Stanford University School of Medicine, summarized his group's studies on the role of insulin in protein synthesis by rat liver. They produced insulin deficiency in two ways—using either alloxan or streptozotocin to destroy the islet cells, or administering anti-insulin serum to neutralize circulating insulin. When animals were studied 48 to 72 hours after the onset of diabetes from the insulin deficiency, protein synthesis was decidedly inhibited regardless of the cause of insulin deficiency. Insulin-deficient liver was found to have a reduced amount of rough endoplasmic reticulum, decreased glycogen content, and enlarged mitochondria. Pronounced differences in protein synthetic activities of the two hepatic ribosomal populations were found. Free ribosomes, unchanged in number, had increased protein synthetic activity. Bound ribosomes were decreased both in number and in protein synthetic activity per milligram of RNA. These changes in bound and free ribosome content and activity were not seen in the livers of growth-hormone deficient diabetic rats. They could also be prevented by insulin administration. The effect of more chronic insulin deficiency on rat liver metabolism was also studied. Protein synthesis by bound ribosomes was restored in seven days; after 28 days it achieved supernormal levels. The protein synthetic activity of free ribosomes remained unchanged in chronic insulin deficiency. The effect of insulin deficiency on hepatic protein synthesis clearly varied with the duration of insulin deficiency.

Donald E. McMillan, MD, director of diabetes research of the Sansum Medical Research Foundation, Santa Barbara, reviewed the potential importance of blood viscosity changes in the development of diabetic microangiopathy. Serum viscosity, increased in both early and late diabetes, is more elevated in diabetic persons who have evidence of microangiopathy—retinopathy, neuropathy, or nephropathy. Extent of microangiopathy is positively correlated with serum viscosity elevation. The major cause of increased serum viscosity is altered serum protein composition. Changes in serum protein synthesis by the liver appear to account for the viscosity increase. Many serum proteins have altered levels in diabetes suggesting that the serum viscosity elevation might be a nonspecific result of altered liver metabolism. When individual protein levels were compared with severity of microangiopathy, it was found that only proteins closely related to viscosity were also directly related to sequelae. The close relation between protein changes increasing viscosity and microangiopathy suggests that altered blood viscosity may play a direct role in producing microcirculatory failure in diabetes. Erythrocyte aggregation is known to be increased in diabetes and the serum viscosity changes may contribute to erythrocyte aggregation. A flow model analysis of the effect of erythrocyte aggregation on blood flow in small tubes indicates that erythrocyte aggregation does not directly impair flow. The viscosity of the peripheral fluid after the aggregates form is lower than that of the original fluid. If erythrocyte aggregation is important in producing diabetic microangiopathy, it must interact to obstruct flow with other factors such as local vessel narrowing.

The following participated in valuable discussions which are not possible to report here: John H. Karam, MD, assistant director, Metabolic Research Unit, University of California, San Francisco School of Medicine; Victor R. Lavis, MD, assistant professor of medicine, University of Washington School of Medicine; Leona V. Miller, MD, associate professor of medicine, University of Southern California School of Medicine; I. Arthur Mirsky, MD, professor of medicine and psychiatry, University of California, Los Angeles School of Medicine; Mark A. Sperling, MD, assistant professor of pediatrics, University of California, Los Angeles School of Medicine; J. C. Sodoyez, MD, research investigator, Orange County Medical Center, Orange; Arne N. Wick, PhD, professor of biochemistry, California State University, San Diego; and Robert H. Williams, MD, professor of medicine, University of Washington School of Medicine.

Perhaps the most impressive element of the conference was the growing diversity of approaches utilized by the investigators in their efforts to improve our understanding of diabetes mellitus and the metabolic and hormonal changes associated with it.